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(54) **Title:** USE OF 3,5 DIIODOTHYRONINE AS REGULATORS OF LIPID METABOLISM

(57) **Abstract:** It has been described a composition including 3,5_T2 in terapeutically efficient doses and diluents and/or vehicles and/or additives pharmaceutically acceptable, to be utilized in all the pre-pathologic and pathologic states related to overweight, and/or obesity, and/or hepatic steatosis alcholic and non-alcholic, and/or dislipidemies, including hypercholesterolemies and hypertryglyceridemies, and/or presence of atherosclerotic plaques and/or hepatopaties associated to dismetabolism, and/or correction of altered lipid metabolism in diabetic subjects, and/or colecistopaties, and/or deposition of undercutaneous fat, including cellulite, and/or vaso motoric rinite, including the allergic one.

USE OF 3,5 DIIODOTHYRONINE AS REGULATORS OF LIPID METABOLISM

Invention field

The invention concerns compositions including 3,5-diiodothyronine and their use in all pre-pathological and pathological states related to accumulation of the lipid component.

Thyroid hormones [(THs); thyroxine (T_4) and 3,3',5-triiodo-L-thyronine (T_3)] are potent stimulator of metabolism and energy expenditure. Most of their effects are exerted through the transcription of genes involved in mechanisms that influence differentiation, growth and metabolism.

The first two actions predominate in the early stages of development of individuals, whereas the metabolic effect predominates in adults.

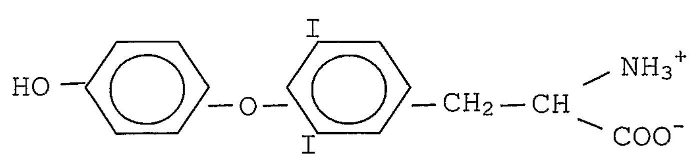
Thyroid hormones are well known both to stimulate metabolism and, at the same time, to lower metabolic efficiency. This last effect has long been the focus of research into the use of THs as drugs to stimulate weight-loss. However, the concomitant induction of a thyrotoxic state has greatly limited the use of these hormones as weight-lowering agents. The term ``thyrotoxicosis'' is used, following L.E Braverman and R.U Utiger (in Werner and Ingbar's- The Thyroid, 47, 667, 2000), to mean the clinical syndrome of hypermetabolism that results when the serum or plasma concentrations of free T_3 (FT_3) and free T_4 (FT_4) are increased as a consequence of the exogenous administration of these substances (the term also includes some pathological

states and the extrathyroidal production of the hormones) and not following an increased thyroid hormones production from the gland (in this case we should use the term hyperthyroidism). In patients the above-mentioned serum parameters constitute the biochemical confirmation of a thyrotoxic state.

Until recently, T_3 was commonly assumed to be the only active hormone with T_4 being its ''precursor''. A growing body of experimental evidences, however, seem to bring to a revision of that opinion and it seems now evident that another iodothyronine, 3,5-diiodo-thyronine or T_2, have biological effects and in particular on metabolism.

Background of the invention

3,5-diiodothyronine (3,5-T2) is a thyroid hormone chemically constituted by two different aromatic rings distinct in inner ring and outer ring. The inner ring (I) harbours a lateral alanine chain whereas the outer ring (O) harbours an hydroxyl group. The carbon atoms of the inner ring are conventionally numbered from 1 to 6 whereas those of the outer ring from 1′ to 6′. In position 3,5 of the inner ring two iodine atoms are present hence the name 3,5-diiodo-thyronine.



Biological actions of 3,5-T2

Recently some authors of the present invention have shown that 3,5-T2 is able to increase mitochondrial respiration

rate and cytochrome oxidase activity when injected into hypothyroid rats. The action is very rapid, and at mitochondrial level it is evidenciable already 1 hour after its injection (Lanni et al., 1992, 1993). A rapid stimulation of mitochondrial respiration, independent of protein synthesis, due to 3,5-T2 has been confirmed by O'Reilly and Murphy (1992); in addition Horst et al. (1989), demonstrated that 3,5-T2, was able to enhance oxygen consumption in perfused rat liver. The perfusion of rat liver with 3,5-T2 induces, in addition, an increase in Calcium uptake inside the mitochondria (Hummerich et al., 1989).

3,5-T2 effects have been shown also in mononuclear human blood cells; the incubation of these cells with 3,5-T2 led to an increased oxygen consumption (Kvetny et al., 1992).

3,5-T2 is able to stimulate hepatic cytochrome oxidase activity (an enzyme whose activity is considered index of the tissue oxidative capacity) both in vivo and in vitro (Lanni et al., 1993, 1994b). Moreover, the addition of 3,5-T2 to cytochrome oxidase complex isolated from bovine heart, induces an increase of activity and a variation of its absorbance spectrum, thus indicating a direct interaction between the above cited substrance and the complex (Goglia et al., 1994). At the mitochondrial level, specific binding sites for 3,5-T2 have been shown in rat liver (Goglia et al., 1994b). The competitions analysis have demonstrated that such sites are highly specific for 3,5-T2 and that others iodothyronines, such as 3,3'-T2, T3, T4 are able to compete significantly only

when present at high concentrations (10^{-5} M). The specific binding is maximal at pH 7.0, temperature of 37 °C, incubation time of 30 min. The presence of mitochondrial binding sites for diiodothyronine suggest an its direct action.

Arnold et al. (1998) by using photoaffinity labelling of cytochrome oxidase (COX) complex, isolated from bovine heart, identified the subunit Va of the COX as the binging site for 3,5-T2.

Kadenbach et al. (2000) demonstrated that 3,5-T2 is able of increasing the respiratory control ratio of a reconstituted COX complex, measured as the ratio of the respiration in the presence of uncouplers over that in their absence. This effect in vitro was seen in the presence of intraliposomal ATP, but not in the presence of ADP, thus under conditions of low energy utilization (high ATP/ADP ratio) the 3,5-T2 induces a redox slip in cytochrome oxidase.

Lanni et al. (1996) and Moreno et al. (1997) have shown that 3,5-T2 is able to increase resting metabolic rate (RMR) of hypothyroid rats, although its effects differed in terms of both time course and dependency on protein synthesis from those of T3.

From such studies it is evident that T_3 increases RMR through a nuclear pathway, as its effect is evident after some days and it is inhibited by the simultaneous injection of actinomycin D (Moreno et al. 1997). The ction of T_2 , on the other hand, is more rapid (its effect on RMR being already evident after 6-16 hours from its single injection) and its independent of protein

synthesis. The effects of T2 are evidenciable in hypothyroid rats but not in euthyroid ones. It has also been demonstrated that T2 is able to stimulate cytosolic enzymes activity such as glucose-6-phosphate dehydrogenase (Lombardi et al., 2000) and to bind specific cytosolic proteins which might play a role of T2 carrier to mitochondria (Moreno et al., 2003).

Description of the invention.

There are not previous data indicating similar effects in normal not hypothyroid rats. The authors administered T2 to normal rats (euthyroid), fed with a hyperlipidic diet . The T2 administration has been done also on 4 human subjects (in a normal euthyroid state) which gave the consensus to the experimentation (A.A., F.G., M.M., A.L.) with a caloric intake 20% above the normal.

One group of rats has been made overweight by feeding with an High Fat Diet (D rats) for 30 days and a subgroup of them was injected with T_2 (DT2 rats). A third group was constituted by normal rats fed with a standard diet (N rats).

On the three groups the following parameters have been evaluated:

-energetic (cellular metabolism),

- body (weight, body fat accumulation, fatty liver, etc.)
- serum parameters related to lipid metabolism (tryglicerides, cholesterol, keton bodies, glycemia, etc.)
- serum thyroid hormone levels (FT3, FT4)

The authors surprisingly found that T2 is able in animals, but also in human subjects, normal (euthyroid) and in the presence of a high fat diet and with an increased food intake, to reduce body weight, and /or to reduce the plasma levels of tryglicerides and cholesterol and /or decrease body fat and/or reduce hepatic steatosis. In a very surprisingly way, there is any induction of a thyrotoxic state, i.e. an increase in FT3 and FT4 serum levels, as indicated by the previous methodology, and it is not appreciable any effect on all the other organs and apparatus. The previous methodological approach has shown that T3 and T4 are able to reduce adipose mass, but with dangerous side effects due to the induced thyrotoxic, state such as tachycardie, ipereccitability, protein catabolism, etc. In addition it has been shown that the increase in metabolism was associated to a loss of body weight due to a loss of lean mass and not of fat mass (Abraham RR et al., 1985. Int. J. Obesity, **9**:433-442; Rozen R et al., 1986. Addictive Behaviors, **10**:303-312, 1986).

The invention resolves the problems of the previous methodological approach offering a composition containing T2 which acts on fat mass and does not have collateral effects, both in experimental animals and in human subjects. The invention thus consists of a composition containing in quantities therapeutically efficient 3,5-diiodothyronine and solvents and/or carriers and /or additives farnaceutically acceptable, to be utilised in all pre-pathologic and patholigic states correlated to overweigth and/or obesity, and/or hepatic

alcoholic and non-alcoholic steatosis, and/or dyslipidemia, including hypercholesterolemia and hypertrigliceridemia and/or the presence of atherosclerotic plaques and/or hepatopathies asociated with dysmetabolism, and/or the correction of altered lipid metabolism in diabetics, and/or cholecystopathies, and/or accumulation of subcutaneous fat, among which for example cellulitis, and/or vaso motoric rinitis, including the allergic variant.

In a particular form of the invention the composition is utilised to reduce body fat mass.

Preferably, the composition consists of 3,5-diiodothyroninea in quantities from 1 to 200 micrograms per kilogram of body weight daily, more preferably 1,5 to 150 micrograms per kilogram of body weight daily, even more preferably from 2 to 90 micrograms per kilogram body weight daily.

The experts in the field will understand that the dosage and administration ways would differ depending on the specific pre.-pathologic or pathologic state and include, but are not limited to dosage forms of oral, parenteral, rectal, nasal, cutaneous administration including rapid and retarded release.

It is otherways understood that the solvents and/or carriers and/or addidives, pharmaceutically acceptable will be selected on the basis of the selected dosage form, like cremes, pomats, drops, injection vials, pills, capsules, rectal pills, inhalatory sprays, plasters, etc.

The invention will now be described in not limitative examples, referred to the following figures:

Fig. 1 Body weight and adipose tissue of N, D and DT2 rats. All rats had the same initial weight (239 ± 5 gr). The animals from group N were fed a standard diet (total metabolizable percentage of energy: 60.4 carbohydrates, 29 proteins, 10.6 fat J/J; 15.88 KJ gross energy/g), the animals from group D were fed with a hyperlipidic diet (total metabolizable percentage of energy: 21 carbohydrates, 29 proteins, 50 fat J/J; 19.85 KJ gross energy/g), and the animals of group DT_2 were fed with a hyperlipidic diet and supplemented with a daily i.p. injection of T_2 (25μg/100g b.w.). **A-** The weight of each rat was measured daily and the data reported are the mean ± ES of 12 animals for each group. **B-** Dorsal views of D and DT2 rats. **C-** Abdominal views of rats, enabling visceral fat pads to be seen. **D-** Visceral fat pads isolated from D and DT_2 rats.

Fig. **2 A-** Histological images of livers obtained from D (left) and DT_2 (right) rats. **B-** Pictures of liver of from D rats with an evident steatosis and from DT2 rats with an evidente absence of fat.

Fig. 3 Plasmatic levels of triglycerides, cholesterol and glucose of N, D and DT2 rats. Data are referred as mean ± ES of 4 animals for each group). *P< 0.05 compared with N value, #P< 0.05 compared with DT_2 value.

Fig. 4 **(A)** Enzymatic activity of total CPT determinated spectrophotometrically from isolated liver mitochondria from N, D and DT2 rats. Data are reported as mean ± ES of 4 animals for each group. * *P*<0.05 compared with N value, ° P< 0.05 compared with D value. **(B)** Proton-leak kinetics of liver mitochondria from N, D and DT_2 rats. Data are

mean ± SEM for 4 rats in each group.

METHODS

Isolation of mitochondrial fraction.

Mitochondrial fraction has been isolated from rat liver. Liver was minced in ice-cold buffer consisting of 220 mM mannitol, 70 mM sucrose, 20 mM Tris-HCl, 1 mM EDTA, and 5 mM EGTA and 5 mM $MgCl_2$, pH 7.4, then homogenized in a Potter-Elvehjem homogenizer. After homogenization, nuclei and cell debris were removed by centrifugation at 500 x g for 10 min, with the resulting supernatant being centrifuged at 3000 x g for 10 min. The mitochondrial pellet was washed twice and resuspended in a minimal volume of isolation medium (final dilution 1:0,5 w/v) and kept on ice. Isolated mitochondria have been used immediately for the determination of respiratory rate and inner mitochondrial membrane potential and for the enzymatic assays.

Determination of protein content

Mitochondrial protein content was determinated by Hartree method (Lowry modified) (Hartree, Anal. Biochem. 48, 422; 1972). This method couple the biuret reaction with the reaction of Folin-Ciocalteu attaining thus a higher sensibility. The developed color is due to the reduction of the phosphotungstic and phosphomolibdic acids into tungsten blue and of molibden by way of the complex of Cu++ protein and of tryptofan and thyrosin in an alcaline environment. The modification with respect to the method of Lowry consists of an increase in the concentration of sodium potassium tartrate and of the use of elevated temperature. This allows to obtain a

calibration curve close to linearity.

Determination of the activity of the COX in the adipose tissue

COX activity was determined polarographycally in adipose tissue homogenates at 25 °C using a Clarck oxygen electrode (Aulie and Gravs, 1979) in a reaction medium containing 30micromolars cytochrome c, 4 micromolars rotenone, 0.5 micromolars DNP, 10 mmolars Na-malonate and 75 millimolars HEPES (pH 7.4). The activity has been measured as the difference between oxygen consumption following addition of the substrate (4millimolar ascorbate, 0.3 millimlar TMPD) and homogenate and the rate of oxygen consumption after addition of the substrate alone in order to take into account the ascorbate autoxidation.

Measurement of mitochondrial respiratory rate and inner membrane mitochondrial potential ($\Delta\Psi$).

For the determination of the variation in proton leak kinetic as a function of $\Delta\Psi$, respiratory rate and membrane potential were measured simultaneously in the same mitochondrial suspension at a temperature of 37 °C. The incubation buffer was constitued by 80 mM KCl, 50 mM Hepes (pH 7.0), 1 mM EGTA, 5 mM KH_2PO_4, 4 mM rotenone, and 1% (w/v) defatted bovine serum albumin (BSA), and 5 mM succinate as substrate as reported by Lombardi et al. (Biochem. J. 330, 521, 1998). The oxygen consumption determination for proton leak measurements has been performed polarographycally by using a Clark-type oxygen electrode, while $\Delta\Psi$ was measued by a triphenylmethylphosphonium ($TPMP^+$)-sensitive electrode.

The electrode specific for TPMP+ allows the measurement in the solution of the activity of the ion by itself. Such electrode is constituted by a thermoplastic resin support at the end of which a TPMP+ selective membrane is applied. Inside to such a system a measurement electrode is placed in a solution containing a known concentration of TPMP+ (10mM) which potential is referred to an external standard electrode. When TPMP+ is added to a mitochondrial suspension, being it charged, is distributed inside and outside of the mitochondrion depending on the $\Delta\Psi$ value. At a steady-state, when $\Delta\Psi$ value is constant, TPMP+ is distributed in a way in which the electrochemical potential of the ion inside and outside the mitochondrial matrix is the same. In such a situation, the TPMP+ follows the equilibrium low of Nernst:

$$\Delta\Psi = \frac{RT}{nF}\ln\frac{TPmP^{+}{}_{\mathrm{int}}}{TPmP^{+}{}_{out}}$$

Where TPMP+int represents the concentration of the ion inside the matrix which is free to diffuse and TPMP+out represents the ion concentration outside the mitochondrion. In effect, the selective electrode is able to measure the value of TPMP+out. The TPMP+int is readly calculated by subtracting TPMP+out from the total quantity of the ion added to the mitochondrial suspension. Sperimentally $\Delta\Psi$ value can be modified by inducing an increase or a decrease in the activity of the reactions which generate it. For example, by varying the concentration of the substrate able to give electrons to the respiratory chain. To this hand, mitochondria were incubated in the respiration medium in the presence of

olygomicine (1mg/ml) (to inhibit proton flux through ATPase), nigerycine (89ng/ml) (to abolish the difference in pH between the inner membrane) and of saturating concentration of succinate able to give the electrons to the respiratory chain (succinate oxidation, via the Krebs cycle, gives rise to FADH2). In such conditions $\Delta\Psi$ and oxygen consumption were determined by progressively additions of malonate, an inhibitor of succinic-dehydrogenase by competing with succinate, (up to a concentration of 2,5 mM), in away to vary electron availability for the respiratory chain and so to determine a reduction in $\Delta\Psi$.

<u>Measurement of fatty acid oxidation rate.</u>

The rate of mitochondrial fatty acid oxidation was assessed polarographically using a Clark-type electrode at 30 °C in a final volume of 0.5 ml of 80 mM KCl, 50 mM Hepes (pH 7.0), 1 mM EGTA, 5 mM K_2HPO_4, 1% BSA (w/v), and 2.5 mM malate in the presence of ADP (120 μg/ml). The reaction was started by the addition of palmitoyl-L-carnitine (40 μM) as reported by Kerner et al. (Am. J. Physiol. 281, E1054, 2001).

<u>Measurement of the total activity of the CPT</u>

Total CPT (CPT1 plus CPT2) activity was measured spectrophotometrically. The spectrophotometric method is based on CoA release from thioesters of Acil-CoA in the presence of DTNB (5,5'-dithio-bis(2-nitrobenzoic acid)) and carnitine which by reduction to 5-thio-2-nitrobenzoic acid develops a yellow color. The reaction is measured following the method reported by Alexson and Nedergaard (J. Biol. Chem. 263, 13564, 1988) by incubation of

mitochondria in: 75 mM HEPES (pH 7.5), 10 mM EDTA, 10 mg/Ml BSA, 2,5 mM palmitoyl-CoA, 3 mM DTNB. All the tubes containing the solutions were incubated at least for 3 min at 35 °C before the addition of palmitoyl-CoA and carnitine. The concentration of the released thiols is calculated by the extension molar coefficient , Σ_{412} value of 13.6 mM^{-1} cm^{-1} after correction by the aspecific reaction of the sulphydril groups of the enzyme with DTNB due to the aspecific hydrolysis of palmitoyl-CoA.

RESULTS

At the end of the treatment period, the D rats were overweight and their weight was about 13% more than rats fed with a standard diet (N rats). While rats treated with T2 showed a strong reduction in their weight (- 13%) and of body fat in comparison with D rats (Fig.1). In fact, DT2 rats accumulated much less fat than the D animals; the visceral fat pad tissue weighed 19.3 ± 2.97 g in D rats and 9.1 ± 2.4 g in DT2 rats. The liver fat content was highly increased in D rats, the hyperlipidic diet thus induced a strong hepatic steatosis which was completely abolished by the T2-treatment (Fig. 2). This data was confirmed by microscopy images, obtained by the pieces inclusion with calcium-phormol followed by Sudan Black B coloration specific for lipid detection (Fig. 2) in which it is evident the complete disapparence of the lipid droplets from the tissue.

Such a result could have been explained by assuming an increased oxidation of fatty acids induced by T2 through the β oxidation. To verify this the mitochondrial β-oxidation rate has been measured in the liver and it has

been shown that it increased in D rats (compared with N) but that in DT2 rats it was furtherly increased; the values in nmoles O/min mg prot were: 61 ± 4 for N, 79 ± 5 for D and 112 ± 7 for DT2 rats. Such an increase in fat utilization should have as a consequence a decrease in serum levels of the parameters related to the ``lipidic state of the animal'' such as :1)tryglicerides, 2) glucose, 3) cholesterol, 4) keton bodies and 5) free fatty acids (NEFA or FFA). The results obtained have shown that, a part from the glucose levels which are unchanged, T2 is able to significatively reduce the cholesterol, FFA and tryglycerides levels (Fig. 3). The keton bodies did not vary as they are used by the muscle tissue instead of fats.

The molecular mechanism underlying these variations include the increased uptake of fatty acids in the cellular compartment of the oxidation (which is the mitochondrion) through the activation of the CPT transporter (carnitine-palmitpyl transferase), but also an inefficient fat utilization. In fact in liver of D rats mitochondria displayed the `` proton-leak'' phenomenon , i.e. a complex mechanism that occurs when energy dispersion as heat is required instead of its storing as body fat (Fig. 4).

At the level of white adipose tissue it has been determined the activity of cytochrome oxidase (an enzyme whose activity is an index of the oxidative capacity of the tissue) and it has been observed an increase of 137% in DT2 rats in comparison with D the values being from 160 ±10 to 380 ± 28 nAtoms O/min mg prot, in D and

DT2 rats. This indicates the capacity of T2 to act directly on adipose tissue reducing its mass.

The same experiments have been performed on humans through voluntaries which gave the permission (A.A., F.G., M.M., A. L.) and which received daily doses of T2 between 15 and 90 micrograms/Kg body weight. The evidenciated effects were:

- a reduction of plasma levels of tryglycerides (from 140 mg/dl to 70 mg/dl) and cholesterol (from 241 mg/dl to 210 mg/dl),
- the plasma parameters (equivalent to the totality of the dosages performed in a laboratory of chemical-clinical analyses) were not influenced by T2
- the resting metabolic rate increased in a dose dependent way reaching a maximum of + 40% (from 1770 Kcal pro die to 2400).
- A reduction in fat mass ranging from 10 to 15% (as measured by impenziometry) with a consequent reduction in body weight.
- A decreased hepatic lipid content (as relieved by echography)
- No significant variations in plasma levels of FT3 and FT4
- No variation in cardiac activity (as relieved by electrocardiography, ecocardiography, and holzer for 24 h)
- Surprisingly, in one of the subject (A.A.) a decrease in motoric vaso rinit was observed.

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CLAIMS

1. Compositions including 3,5-diiodothyronine in quantities terapeutically efficient and diluents and/or vehicles and/or additives pharmaceutically acceptable, to be utilized in all the pre-pathologic and pathologic states related to overweight, and/or obesity, and/or hepatic steatosis alcholic and non-alcholic, and/or dislipidemies, inc luding hypercholesterolemies and hypertryglyceridemies, and/or presence of atherosclerotic plaques and/or hepatopaties associated to dismetabolism, and/or corrction of altered lipid metabolism in diabetic subjects, and/or colecistopaties, and/or deposition of undercutaneous fat, including cellulitis, and/or vaso motoric rinite, including the allergic one.
2. Composition according to rivendication 1 for the reduction of body fat mass.
3. Composition according the previous cited rivendications constituted by 3,5-T2 in a dosage from 1 microgram to 200 micrograms/Kg body weight, daily.
4. Composition according to rivendication 3 constituted by 3,5-T2 in a dosage from 1,5 microgram to 150 micrograms/Kg body weight, daily.
5. Composition according to rivendication 4 constituted by 3,5-T2 in a dosage from 2 microgram to 90 micrograms/Kg body weight, daily.
6. Use of 3,5-T2 for the preparation of a composition to be utilised in all the pre-pathologic and pathologic states related to overweight, and/or obesity, and/or hepatic steatosis alcholic and non-

alcholic, and/or dislipidemies, including hypercholesterolemies and hypertrygly- ceridemies, and/or presence of atherosclerotic plaques and/or hepatopaties associated to dismetabolism, and/or corrction of altered lipid metabolism in diabetic subjects, and/or colecistopaties, and/or deposition of undercutaneous fat, including cellulitis, and/or vaso motoric rhinitis, including the allergic one.

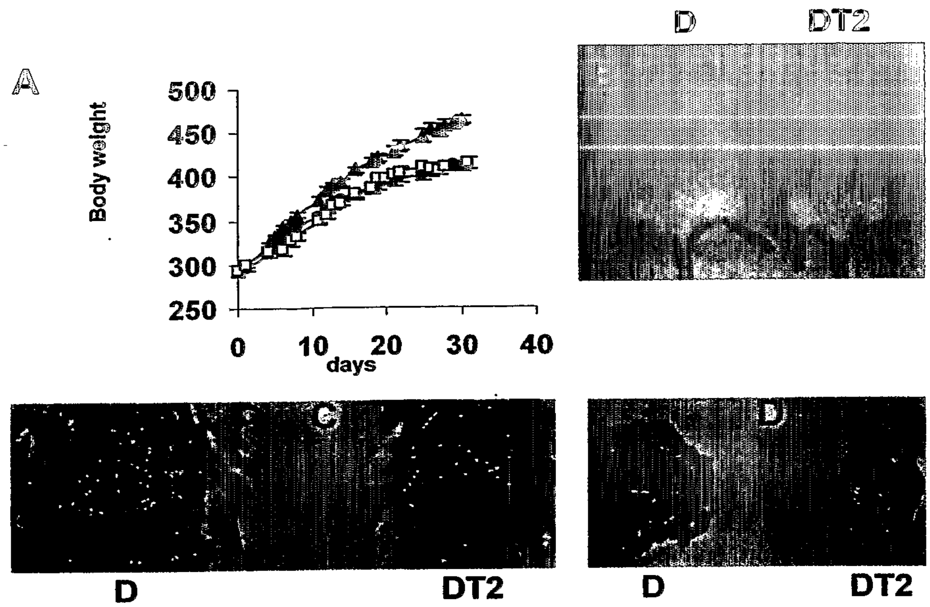

A
Body weight
500
450
400
350
300
250
0
10
20
30
40
days
D
DT2
C
D
DT2
D
DT2

FIG. 1

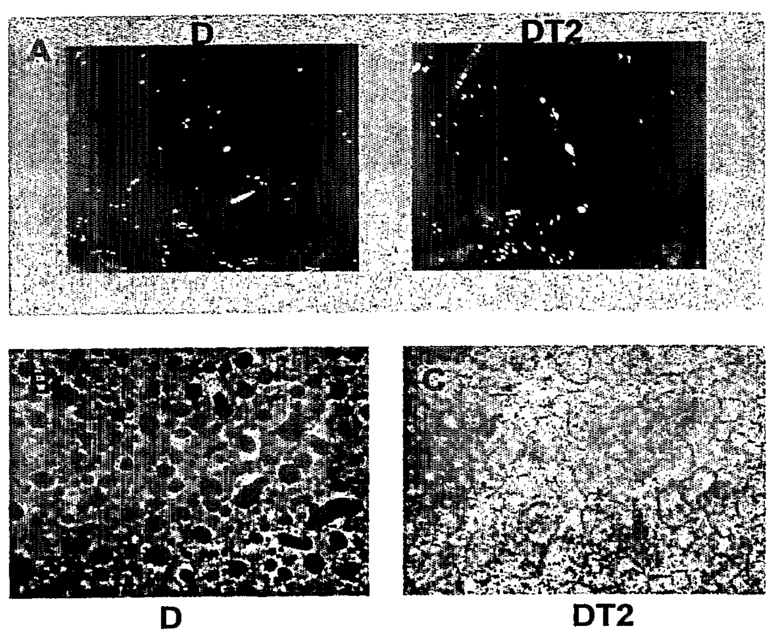

A
D
DT2
B
C
D
DT2

FIG. 2

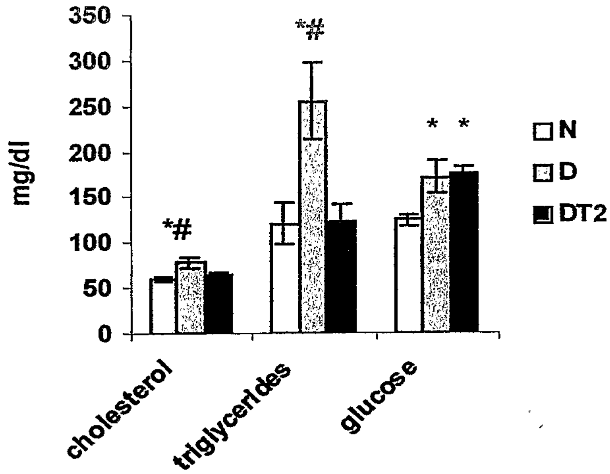
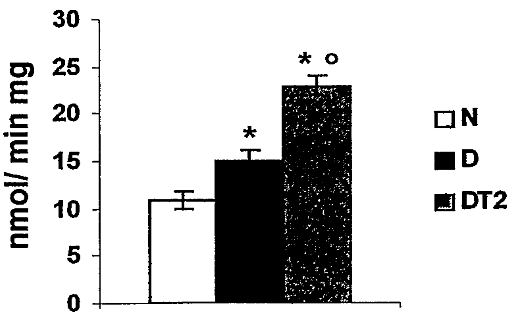

350
300
250
200
150
100
50
0
mg/dl
*#
*#
* *
N
D
DT2
cholesterol
triglycerides
glucose

FIG. 3

A


30
25
20
15
10
5
0
nmol/ min mg
*
* o
N
D
DT2

B

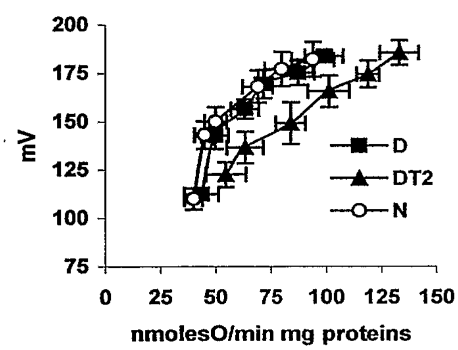

200
175
150
125
100
75
mV
0 25 50 75 100 125 150
nmolesO/min mg proteins
D
DT2
N

FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IT2004/000402

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/198 A61P5/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ, WPI Data, EMBASE, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 October 2004	16/11/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Giacobbe, S

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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PCT/IT2004/000402

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